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The pharmacokinetics and tissue distribution of coumaroylspinosin in rat: A novel flavone C-glycoside derived from Zizyphi Spinosi Semen

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ABSTRACT

Zizyphi Spinosi Semen (ZSS) has a long history of sedative-hypnotic use in China. As a novel flavone C-glycoside, coumaroylspinosin is a main flavonoid only found in ZSS. Up to now, its pharmacokinetic information and tissue distribution *in vivo* are not available yet. With a simple, rapid and sensitive HPLC–MS/MS method, the pharmacokinetics and tissue distribution of coumaroylspinosin were investigated in Sprague-Dawley (SD) rats after its intravenous administration. Puerarin was used as the internal standard (IS). The samples were extracted by a simple protein precipitation method with methanol. The MS analysis was performed with multiple reaction monitoring (MRM), and the transitions were set at m/z 753.3 → 427.0 for coumaroylspinosin and m/z 415.3 → 295.3 for IS, respectively. The method was successfully applied for investigating the pharmacokinetics and tissue distribution of coumaroylspinosin in Sprague Dawley (SD) rats after tail vein injection with 4.0 mg/kg of the flavonoid. The calibration curves covered over the range of 0.02–10 μg/mL in plasma and various tissues samples with good linearity($r \geq 0.9956$). The lower limit of quantification (LLOQ) in all samples was less than 20 ng/mL. The intra- and inter-day precisions were below 15% and accuracy was from −3.78% to 4.68%. No significant matrix effect was observed, and the average extraction recovery was acceptable. Coumaroylspinosin could be cleared quickly from the rat plasma with the half-life ($t_{1/2}$) of 1.86 ± 0.15 h. It was distribute widely *in vivo*, and the main tissue depots of coumaroylspinosin in rats were found to be intestine, muscle and lung. With the method, the pharmacokinetic parameters and tissue distribution of coumaroylspinosin in SD rats were investigated for the first time. The results demonstrated that coumaroylspinosin was distributed widely and rapidly in various rat tissues after intravenous administration.

1. Introduction

Zizyphi Spinosi Semen (ZSS), the ripe and dried seeds of *Zizyphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex H. F. Chou, has a long history of medicinal use for nourishing the liver, calming the mind, and soothing the nerves in the traditional Chinese medicine [1,2]. In the recent years, much pharmacological research has demonstrated that ZSS exerts various bioactivities, such as sedative-hypnotic [3,4], memory modulating [5], and anti-anxiety effects [6].

Apart from triterpenoid saponins, flavonoids have been demonstrated to be the main constituents responsible for the specific bioactivities of ZSS [7,8]. Up to now, over 20 flavonoid components have been isolated and identified in ZSS, including spinosin, zivulgarin, 6‴-feruloylspinosin, 6-sinapoylspinosin, swertisin [9,10], and so on. Due to its high content in ZSS (no less than 0.08%, w/w), spinosin is considered as a key active ingredient in evaluating the quality of this herb [11]. It displayed great potential as a new natural product for improving sleep [12], ameliorating memory [13,14] and anti-anxiety [15].

Coumaroylspinosin is a novel flavone C-glycoside, which has only been found in ZSS up to now [16]. It is a derivative of spinosin,

Abbreviations: AUC, area under the curve; CE, collision energy; CL, clearance rate; ESI, electrospray ionization; IS, internal standard; LLOQ, lower limit of quantification; MRM, multiple reaction monitoring; MRT, mean residence time; QC, quality control; RE, relative error; RSD, relative standard deviation; SD rat, Sprague Dawley rat; ZSS, Zizyphi Spinosi Semen.

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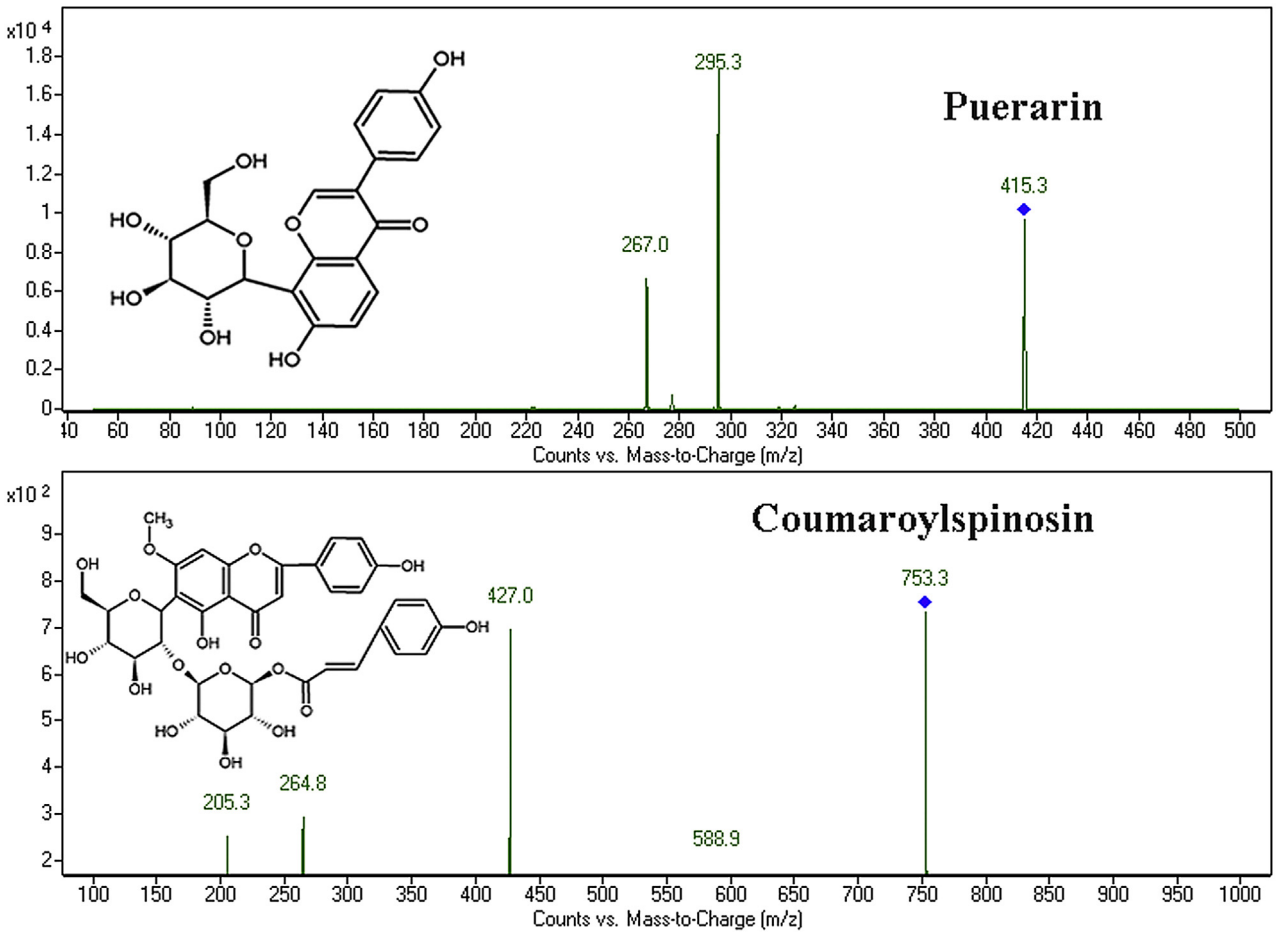

Fig. 1. The chemical structures and mass spectra of coumaroylspinosin and IS.

in which a coumaroyl is bound to 6‴-C of the glycoside (Fig. 1). Although coumaroylspinosin is also a main flavonoid in ZSS [17], its pharmacokinetic information and tissue distribution *in vivo* are not available yet.

In the present study, with a simple, rapid and sensitive HPLC–MS/MS method, the pharmacokinetics and tissue distribution of coumaroylspinosin were investigated in SD rats after intravenous administration of the flavonoid. Our results would provide useful information for the further development of coumaroylspinosin in its clinical application.

2. Materials and methods

2.1. Chemicals and materials

Coumaroylspinosin ([(2*R*,3*S*,4*S*,5*R*,6*S*)-6-[(2*S*,3*R*,4*S*,5*S*,6*R*)-4,5-dihydroxy-2-[5-hydroxy-2-(4-hydroxyphenyl)-7-methyl-4-oxo-chromen-6-yl]-6-(hydroxymethyl)oxan-3-yl]oxy-3,4,5-trihydroxy-oxan-2-yl]methyl(*E*)-3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoate, $C_{38}H_{40}O_{17}$, purity over 98%) was prepared in our laboratory. The procedure was summarized as follows: ZSS were dried and ground into powder (40 meshes). After being refluxed with petroleum ether (60–90 °C) to remove the lipids, the residue was extracted with 70% ethanol. The extract was further purified by an AB-8 macroporous adsorption resin column, and 45% ethanol eluent was collected to be the crude flavonoids. It was then chromatographed on Sephadex LH-20 with 30% methanol as the eluent, and a single peak was obtained. Through comparing its UV, MS, ^{1}H NMR and chromatographic behaviors with the reported data [17], the compound was confirmed to be coumaroylspinosin.

Puerarin (internal standard, IS) was purchased from Chengdu Biological Technology Co., Ltd (Sichuan, China). HPLC grade methanol, acetonitrile and water were all purchased from J. T. Baker (J. T. Baker Chemicals, USA). Formic acid was from TEDIA (TEDIA, USA). All of the other chemicals were of analytical grade.

2.2. Animals

Male SD rats (200 ± 20 g) were purchased from Tianjin Institute of Materia Medica (Tianjin, China). All the rats were acclimated for 5 days before the treatment, and received a standard chow diet (including water) *ad libitum* in environmentally controlled cages (12 h light/dark cycle and relative humidity 50%). Before the experiments, all the rats were fasted overnight (free access to water) [18,19]. The study was conducted in accordance with the National Guidelines on the Proper Care and Use of Animals in Laboratory Research. The experimental protocols were approved and guided by the local Animals Ethical Committee of Tianjin University of Commerce.

2.3. HPLC and MS/MS conditions

Agilent 1200 series systems (Agilent Technologies, US) were used in this research, including a G1367D autosampler SL Plus, a G1312B binary pump, a G1316B column heater and a G1322A vacuum degasser. The separation was performed on a YMC ODS-AQ^{TM} column (2.0 × 250 mm, 5 μm) with a mobile phase consisting of